

WHAT IS CLAIMED IS:

1. A method of identifying differences in nucleic acid levels between two or more nucleic acid samples, said method comprising the steps of:

(a) providing one or more oligonucleotide arrays said arrays comprising probe oligonucleotides attached to a surface;

(b) hybridizing said nucleic acid samples to said one or more arrays to form hybrid duplexes between nucleic acids in said nucleic acid samples and probe oligonucleotides in said one or more arrays that are complementary to said nucleic acids or subsequences thereof;

(c) contacting said one or more arrays with a nucleic acid ligase; and

(d) determining differences in hybridization between said nucleic acid samples wherein said differences in hybridization indicate differences in said nucleic acid levels.

2. The method of claim 1, further comprising contacting said oligonucleotide arrays with one or more ligatable oligonucleotides.

3. The method of claim 2, wherein said ligatable oligonucleotides are a pool of all possible oligonucleotides of a preselected length.

4. The method of claim 2, wherein said determining comprises detecting one or more of said ligatable oligonucleotides attached to said array.

5. The method of claim 1, wherein said one or more arrays is at least two arrays and said arrays are essentially the same in probe oligonucleotide composition.

6. The method of claim 5, wherein the spatial arrangement of said probe oligonucleotides is essentially the same in said arrays.

7. The method of claim 1, wherein each of said nucleic acid samples is hybridized to a different array, the different arrays having substantially the same probe oligonucleotide composition.

8. The method of claim 1, wherein two or more of said nucleic acid samples are hybridized to a single oligonucleotide array.

9. The method of claim 8, wherein said nucleic acid samples are simultaneously hybridized to a single oligonucleotide array.

10. The method of claim 1, wherein said probe oligonucleotides are pairs of probe oligonucleotides that differ from each other in preselected nucleotides.

11. The method of claim 10, wherein said pairs of probe oligonucleotides differ from each other in a single nucleotide.

12. The method of claim 10, wherein said determining comprises determining the difference in sample nucleic acid hybridization intensity between the members of said pairs of probe oligonucleotides.

13. A method of identifying differences in nucleic acid levels between two or more nucleic acid samples, said method comprising the steps of:

(a) providing one or more oligonucleotide arrays comprising probe oligonucleotides wherein said probe oligonucleotides comprise a constant region and a variable region;

(b) hybridizing said nucleic acid samples to said one or more arrays to form hybrid duplexes between nucleic acids in said nucleic acid samples and said variable regions that are complementary to said nucleic acids or subsequences thereof; and

(c) determining differences in hybridization between said nucleic acid samples wherein said differences in hybridization indicate differences in said nucleic acid levels.

14. The method of claim 13, wherein said variable region varies in length from about 3 nucleotides to about 50 oligonucleotides.

15. The method of claim 13, wherein the variable regions of said probe oligonucleotides comprise all possible oligonucleotides of a preselected length.

16. The method of claim 15, wherein said variable regions are at least 5 nucleotides in length.

17. The method of claim 13, wherein said constant region ranges in length from 3 nucleotides to about 25 nucleotides.

18. The method of claim 13, wherein said constant regions comprise a nucleotide sequence complementary to a sense or antisense sequence of the recognition site of a restriction endonuclease.

19. The method of claim 13, further comprising contacting said oligonucleotide arrays with a constant oligonucleotide complementary to said constant region or a subsequence thereof.

20. The method of claim 19, comprising contacting said array with a ligase.

21. The method of claim 19, wherein said determining comprises detecting a nucleic acid of said nucleic acid samples attached to said constant oligonucleotide.

22. The method of claim 13, wherein said probe oligonucleotides are pairs of probe oligonucleotides that differ from each other in preselected nucleotides.

23. The method of claim 22, wherein said determining comprises determining the difference in sample nucleic acid hybridization intensity between the members of said pairs of probe oligonucleotides.

24. A method of identifying differences in nucleic acid levels between two or more nucleic acid samples, said method comprising the steps of:

(a) providing one or more arrays of oligonucleotides each array comprising pairs of probe oligonucleotides where the members of each pair of probe oligonucleotides differ from each other in preselected nucleotides;

(b) hybridizing said nucleic acid samples to said one or more arrays to form hybrid duplexes between nucleic acids in said nucleic acid samples and

probe oligonucleotides in said one or more arrays that are complementary to said nucleic acids or subsequences thereof:

(c) determining the differences in hybridization between said nucleic acid samples wherein said differences in hybridization indicate differences in said nucleic acid levels.

25. The method of claim 24, wherein said members of each pair of probe oligonucleotides differ from each other in a centrally located nucleotide.

26. A method of identifying differences in nucleic acid levels between two or more nucleic acid samples, said method comprising the steps of:

(a) providing one or more arrays of oligonucleotide arrays each array comprising more than 100 different probe oligonucleotides wherein:

each different probe oligonucleotide is localized in a predetermined region of the array;

each different probe oligonucleotide is attached to a surface through a terminal covalent bond;

the density of said probe different oligonucleotides is greater than about 60 different oligonucleotides per 1 cm<sup>2</sup>;

(b) hybridizing said nucleic acid samples to said one or more arrays to form hybrid duplexes between nucleic acids in said nucleic acid samples and probe oligonucleotides in said one or more arrays that are complementary to said nucleic acids or subsequences thereof;

(c) determining the differences in hybridization between said nucleic acid samples wherein said differences in hybridization indicate differences in said nucleic acid levels.

27. The method of claim 26, further comprising contacting said one or more oligonucleotide arrays with a ligase.

28. A method of identifying differences in nucleic acid levels between two or more nucleic acid samples, said method comprising the steps of:

(a) providing one or more oligonucleotide arrays each comprising probe oligonucleotides wherein said probe oligonucleotides are not chosen to hybridize to nucleic acids derived from particular preselected genes or mRNAs;

(b) hybridizing said nucleic acid samples to said one or more arrays to form hybrid duplexes between nucleic acids in said nucleic acid samples and probe oligonucleotides in said one or more arrays that are complementary to said nucleic acids or subsequences thereof; and

(d) determining differences in hybridization between said nucleic acid samples wherein said differences in hybridization indicate differences in said nucleic acid levels.

29. The method of claim 28, wherein said probe oligonucleotides are pairs of probe oligonucleotides that differ from each other in preselected nucleotides.

30. The method of claim 29, wherein said determining comprises determining the difference in sample nucleic acid hybridization intensity between the members of said pairs of probe oligonucleotides.

31. A method of identifying differences in nucleic acid levels between two or more nucleic acid samples, said method comprising the steps of:

(a) providing one or more oligonucleotide arrays each comprising probe oligonucleotides wherein said probe oligonucleotides comprise a nucleotide sequence or subsequences selected according to a process selected from the group consisting of a random selection, a haphazard selection, a nucleotide composition biased selection, and all possible oligonucleotides of a preselected length;

(b) hybridizing said nucleic acid samples to said one or more arrays to form hybrid duplexes between nucleic acids in said nucleic acid samples and probe oligonucleotides in said one or more arrays that are complementary to said nucleic acids or subsequences thereof; and

(c) determining differences in hybridization between said nucleic acid samples wherein said differences in hybridization indicate differences in said nucleic acid levels.

32. The method of claim 31, wherein said nucleotide sequence or nucleotide subsequences are all possible oligonucleotides of a preselected length selected from the group consisting of: all possible 6 mers, all possible 7 mers, all

possible 8 mers, all possible 9 mers, all possible 10 mers, all possible 11 mers, and all possible 12 mers.

33. A method of simultaneously monitoring the expression of a multiplicity of genes, said method comprising:

- (a) providing a pool of target nucleic acids comprising RNA transcripts of one or more of said genes, or nucleic acids derived from said RNA transcripts;
- (b) hybridizing said pool of nucleic acids to an oligonucleotide array comprising probe oligonucleotides immobilized on a surface;
- (c) contacting said oligonucleotide array with a ligase; and
- (d) quantifying the hybridization of said nucleic acids to said array wherein said quantifying provides a measure of the levels of transcription of said genes.

34. The method of claim 33, wherein said probe oligonucleotides comprise nucleotide sequences or nucleotide subsequences complementary to preselected RNA transcripts of one or more of said genes, or nucleic acids derived from said RNA transcripts.

35. A method of simultaneously monitoring the expression of a multiplicity of genes, said method comprising:

- (a) providing one or more oligonucleotide arrays comprising probe oligonucleotides wherein said probe oligonucleotides comprise a constant region and a variable region;
- (b) providing a pool of target nucleic acids comprising RNA transcripts of one or more of said genes, or nucleic acids derived from said RNA transcripts;
- (c) hybridizing said pool of nucleic acids to said array of oligonucleotide probes; and
- (d) quantifying the hybridization of said nucleic acids to said array wherein said quantifying provides a measure of the levels of transcription of said genes.

36. The method of claim 35, wherein said probe oligonucleotides comprise nucleotide sequences or nucleotide subsequences complementary to

preselected RNA transcripts of one or more of said genes, or nucleic acids derived from said RNA transcripts.

37. A method of making a nucleic acid array for identifying differences in nucleic acid levels between two or more nucleic acid samples, said method comprising the steps of:

(a) providing an oligonucleotide array comprising probe oligonucleotides wherein said probe oligonucleotides comprise a constant region and a variable region;

(b) hybridizing one or more of said nucleic acid samples to said arrays to form hybrid duplexes of said variable region and nucleic acids in said nucleic acid samples comprising subsequences complementary to said variable region;

(c) attaching the sample nucleic acids comprising said hybrid duplexes to said array of probe oligonucleotides; and

(d) removing unattached nucleic acids to provide a high density oligonucleotide array bearing sample nucleic acids attached to said array.

38. A method of making a nucleic acid array for identifying differences in nucleic acid levels between two or more nucleic acid samples, said method comprising the steps of:

(a) providing an array comprising more than 100 different probe oligonucleotides wherein:

each different probe oligonucleotide is localized in a predetermined region of the array;

each different probe oligonucleotide is attached to a surface through a terminal covalent bond;

the density of said probe different oligonucleotides is greater than about 60 different oligonucleotides per  $1 \text{ cm}^2$ ;

(b) contacting said array one or more of said two or more nucleic acid samples whereby nucleic acids of said one of said two or more nucleic acid samples form hybrid duplexes with probe oligonucleotides in said arrays;

(c) attaching the sample nucleic acids comprising said hybrid duplexes to said array of probe oligonucleotides; and

(d) removing unattached nucleic acids to provide a high density oligonucleotide array bearing sample nucleic acids attached to said array.

39. A kit for identifying differences in nucleic acid levels between two or more nucleic acid samples, said kit comprising:

- a container containing one or more oligonucleotide arrays said arrays comprising probe oligonucleotides attached to a surface; and
- a container containing a ligase.

40. A kit for identifying differences in nucleic acid levels between two or more nucleic acid samples, said kit comprising:

- a container containing one or more oligonucleotide arrays said arrays comprising probe oligonucleotides wherein said probe oligonucleotides comprise a constant region and a variable region/

41. The kit of claim 40, further comprising a constant oligonucleotide complementary to said constant region or a subsequence thereof.

42. A method of labeling a nucleic acid, said method comprising the steps of:

- (a) providing a nucleic acid;
- (b) amplifying said nucleic acid to form amplicons;
- (c) fragmenting said amplicons to form fragments of said amplicons; and
- (d) coupling a labeled moiety to at least one of said fragments.

43. A method of labeling a nucleic acid, said method comprising the steps of:

- (a) providing a nucleic acid;
- (b) transcribing said nucleic acid to form a transcribed nucleic acid;
- (c) fragmenting said transcribed nucleic acid to form fragments of said transcribed nucleic acid; and
- (d) coupling a labeled moiety to at least one of said fragments.

44. A method of labeling a nucleic acid comprising the steps of:

- (a) providing at least one nucleic acid coupled to a support;

(b) providing a labeled moiety capable of being coupled with a terminal transferase to said nucleic acid;

(c) providing said terminal transferase; and

(d) coupling said labeled moiety to said nucleic acid using said terminal transferase.

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~~44~~<sup>45</sup> A method of labeling a nucleic acid comprising the steps of:

(a) providing at least two nucleic acids coupled to a support;

(b) increasing the number of monomer units of said nucleic

acids to form a common nucleic acid tail on said at least two nucleic acids;

(c) providing a labeled moiety capable of recognizing said common nucleic acid tails; and

(d) contacting said common nucleic acid tails and said labeled moiety.

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~~45~~<sup>46</sup> A method of labeling a nucleic acid comprising the steps of:

(a) providing at least one nucleic acid coupled to a support;

(b) providing a labeled moiety capable of being coupled with a ligase to said nucleic acid;

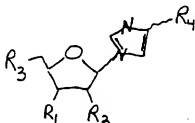
(c) providing said ligase; and

(d) coupling said labeled moiety to said nucleic acid using said ligase.

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~~46~~<sup>47</sup> A compound having the formula:



wherein R1 is hydrogen, hydroxyl, a phosphate linkage, or a phosphate group;

R2 is hydrogen or hydroxyl;

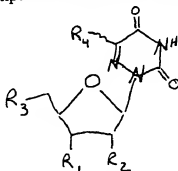
R3 is hydrogen, hydroxyl, a phosphate linkage, or a phosphate group; and

R4 is a coupled labeled moiety.

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A compound having the formula:



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wherein R1 is hydrogen, hydroxyl, a phosphate linkage, or a phosphate group;  
R2 is hydrogen or hydroxyl;  
R3 is hydrogen, hydroxyl, a phosphate linkage, or a phosphate group; and  
R4 is a coupled labeled moiety.

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A method of identifying differences in nucleic acid levels between two or more nucleic acid samples, said method comprising the steps of:

(a) providing one or more oligonucleotide arrays each comprising probe oligonucleotides wherein said probe oligonucleotides comprise a nucleotide sequence or subsequences selected according to a process selected from the group consisting of a random selection, a haphazard selection, a nucleotide composition biased selection, and all possible oligonucleotides of a preselected length;

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(b) providing software describing the location and sequence of probe oligonucleotides on said array;

(c) hybridizing said nucleic acid samples to said one or more arrays to form hybrid duplexes between nucleic acids in said nucleic acid samples and probe oligonucleotides in said one or more arrays that are complementary to said nucleic acids or subsequences thereof;

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(d) operating said software such that said hybridizing indicates differences in said nucleic acid levels.